EXPRESS MAIL MAILING LABEL NO. EL917371535US

PATENT

Attorney Docket No. INL-044C1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Rosén et al.		
SERIAL NO.:	Not yet assigned	GROUP NO.:	Not yet assigned
FILING DATE:	Herewith	EXAMINER:	Not yet assigned
TITLE:	IN VITRO METHODS FOR SCREENING FOR BLOOD COAGULATION DISORDERS USING METAL IONS (AS AMENDED)		

BOX PATENT APPLICATION
Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please make the following amendments prior to examination.

In the Title

Please amend the title to read --In Vitro Methods for Screening for Blood Coagulation Disorders Using Metal Ions--.

In the Specification

On page 1, please replace paragraph 1, lines 4-5, with the following:

--This is a continuation of U.S. Application No. 09/273,413 filed March 19, 1999, which claims priority to European Appln. 98105043.8, filed on March 19, 1998, the disclosures of which are incorporated herein by reference.--

In the Drawings

Please enter the formal drawings in which no text has been added or removed, but rather, the margins and figure legends have been corrected in accordance with the Notice of Draftsperson's Patent Drawing Review (copy enclosed) that issued in the parent application. A Letter to the

Draftsperson requesting entry of the enclosed formal drawings is also enclosed.

In the Claims

Please cancel claims 1-44, and add new claims 45-84 as follows.

45. An in vitro method determining the functional activity of one or more components of the Protein C anticoagulant pathway of the blood coagulation system, comprising:

(a) providing a blood sample to be analyzed;

(b) activating the coagulation cascade by adding a procoagulant reagent to the blood sample to be analyzed;

(c) triggering coagulation by adding calcium ions to the blood sample;

(d) adding metal ions selected from the group consisting of Mn^{+2}, Zn^{+2}, Ni^{+2}, Sr^{+2}, Cu^{+2}, or Cu^{+} ions at a concentration that increases the anticoagulant activity of one or more components of the Protein C anticoagulant pathway;

(e) incubating a reaction mixture comprising the components recited in steps (a)-(d);

(f) observing clotting time; and

(g) comparing the clotting time for the blood sample to be analyzed with the clotting time for a normal blood sample as determined by the method recited in steps (a)-(f).

46. The method according to claim 45, wherein the metal ion is Mg^{2+}.

47. The method according to claim 46, wherein the amount of the Mg^{2+} ions added is about 20 μmol to 10 mmol per liter of reaction mixture.

48. The method according to claim 46, wherein the amount of the Mg^{2+} ions added in step (d) is about 100 μmol to 2 mmol per liter of reaction mixture.

49. The method according to claim 46, wherein the amount of the Mg^{2+} ions added in step (d) is about 200 μmol to 1 mmol per liter of reaction mixture.

50. The method according to claim 45, wherein the amount of Mn^{+2}, Zn^{+2}, Ni^{+2}, Sr^{+2}, Cu^{+2}, or Cu^{+} ions added in step (d) is about 1 μmol to 2 mmol per liter of reaction mixture.

51. The method according to claim 45, wherein the amount of Mn^{+2}, Zn^{+2}, Ni^{+2}, Sr^{+2}, Cu^{+2}, or Cu^{+} ions added in step (d) is about 5 μmol to 400 μmol per liter of reaction mixture.

52. The method according to claim 45, wherein the amount of Mn^{+2}, Zn^{+2}, Ni^{+2}, Sr^{+2}, Cu^{+2}, or Cu^{+} ions added in step (d) is about 10 μmol to 80 μmol per liter of reaction mixture.

53. The method according to claim 45, wherein the blood sample is selected from the group consisting of whole blood, blood plasma, and blood serum.

54. The method according to claim 45, wherein the activating, triggering, and adding steps occur separately.

55. The method according to claim 45, wherein the activating, triggering, and adding steps occur simultaneously.

56. The method according to claim 45, wherein the amount of calcium ions added in step (c) is about 0.5 mmol to 20 mmol per liter of reaction mixture.

57. The method according to claims 45, wherein the amount of calcium ions added in step (c) is about 1 mmol to 10 mmol per liter of reaction mixture.

58. The method according to claims 45, wherein the amount of calcium ions added in step (c) is about 200 μmol to 1 mmol per liter of reaction mixture.

59. The method according to claims 45, wherein the activating the coagulation cascade step occurs via the intrinsic pathway and the coagulation activator for the intrinsic pathway compositions comprises phospholipid(s) and contact activators.

60. The method according to claim 45, wherein the activating the coagulation cascade step occurs via the intrinsic pathway and the coagulation activator comprises phospholipids and an intrinsic pathway factor selected from the group consisting of Factor IXa, Factor XIIa, and Factor XIa.

61. The method according to claim 45, wherein the activating the coagulation cascade step occurs via the intrinsic pathway and the coagulation activator and the coagulation activator comprises an intrinsic pathway factor selected from the group consisting of Factors IXa, Factor XIIa, and Factor XIa.

62. The method according to claim 59, wherein the contact activator is selected from the group consisting of ellagic acid, collagen, collagen-related substances, and silica.

63. The method according to claim 62, wherein the contact activator is a silica selected from the group consisting selected from the group consisting of micronized silica, colloidal silica, and kaolin.

64. The method according to claim 45, wherein the activating the coagulation cascade step occurs via the extrinsic pathway and the coagulation activator for the extrinsic pathway is selected from the group consisting of native human tissue factor, recombinant human tissue factor, non-human native tissue factor, non-human recombinant tissue factor, native human Factor VII/VIIa, recombinant human Factor VII/VIIa, native non-human Factor VII/VIIa, and recombinant non-human Factor VII/VIIa.

65. The method according to claims 59-60, wherein the phospholipids are selected from the group consisting of synthetic phospholipids, purified phospholipids, and crude extracts of phospholipids derived from biological sources.

66. The method according to claim 66, wherein the phospholipids are selected from the group consisting of phosphatidylcholine, phosphatidylserine, and sphinogmyelin.

67. The method according to claim 45, wherein the activating the coagulation cascade step occurs via the common pathway and the coagulation activator is selected from the group consisting of exogenous Factor Xa, exogenous Factor X and an exogenous activator for Factor X, and an exogenous activator for endogenous Factor X.

68. The method according to claim 67, wherein the exogenous activator for Factor X is snake venom enzyme.

69. The method according to claim 68, wherein the exogenous activator for Factor X comprises Russelli Viperii snake venom enzyme.

70. The method according to claim 45, further comprising the step of adding components of the Protein C anticoagulant pathway to compensate for variable functional levels of the components of the anticoagulant pathway in the sample, said components being selected from the group consisting of Protein C, activated Protein C, Protein S, Factor V, Factor Va, a plasma deficient of the Protein C anticoagulant pathway component to be analyzed, and a plasma deficient of all components of the Protein C anticoagulant pathway.

71. The method according to claim 45, further comprising the step of adding a fibrin polymerization inhibitor to the blood sample to be analyzed.

72. The method according to claim 71, wherein the fibrin polymerization inhibitor comprises Gly-Pro-Arg-Pro.

73. The method according to claim 45, wherein the procoagulation reagent is selected from the group consisting of Factor VIII, Factor VIIIa, Factor IX, Factor X, and prothrombin.

74. The method of claim 45, wherein the component of the Protein C anticoagulant pathway analyzed is Protein C, said method further comprising the step of activating Protein C by adding exogenous activated Protein C to the blood sample to be analyzed.

75. The method according to claim 45, wherein the component of the Protein C anticoagulant pathway analyzed is Protein C, said method further comprising the step of activating Protein C by adding an activator of Protein C to the blood sample to be analyzed.

76. The method according to claim 45, wherein the component of the Protein C anticoagulant pathway analyzed is Protein C, said method further comprising the step of adding exogenous Protein C together with an activator of Protein C to the blood sample to be analyzed.

77. The method according to claim 45, wherein the metal ions are added simultaneously with the Protein C activator.

78. The method according to claim 45, wherein the activating Protein C step occurs simultaneously with activating the coagulation cascade step.

79. The method according to claim 45, wherein the activating Protein C step precedes the activating the coagulation cascade step

80. The method according to claim 45, wherein the activator for Protein C comprises Protein C activating snake venom enzyme and thrombin.

81. The method according to according to claim 45, wherein the activator for Protein C comprises thrombomodulin.

82. The method according to claim 80, wherein the Protein C activator is recombinant Protein C activator.

83. The method according to according to claim 82, wherein the Protein C activating snake venom enzyme is obtained from the Agkistrodon family of Agkistrodon contortrix contortrix.

84. The method according to claim 83, wherein the amount of purified Protein C activator added is about 2×10^{-3} U to 0.3 U per milliliter of reaction mixture.

REMARKS

Claims 1-45 are cancelled and claims 45-84 are added by the present Preliminary Amendment. Applicants submit that no new matter is added.

Applicants believe that no additional fees are necessitated by this Preliminary Amendment. However, in the event that any additional fees are due, the Commissioner is hereby authorized to charge Deposit Account No. 20-0531.

Respectfully submitted,

Maureen A. Bresnahan

Date: January 16, 2002
Reg. No. 44,559
Tel. No. (617) 248-7103
Fax No. (617) 248-7100

Maureen A. Bresnahan
Attorney for Applicants
Testa, Hurwitz & Thibeault, LLP
High Street Tower
125 High Street
Boston, MA 02110

2260069

MARKED-UP VERSION OF AMENDED PARAGRAPHS

Marked-up version of paragraph 1, page 1:

This is a continuation of U.S.S.N. 09/273,413 filed March 19, 1999, which claims priority to European Appln. 98105043.8, filed on March 19, 1999, the disclosures of which are incorporated herein by reference. [This application claims priority to European Pat. Appln. 98 105 043.8, filed March 19, 1998.]